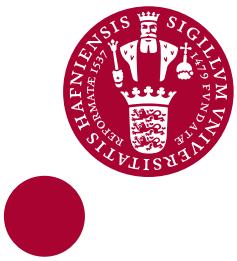

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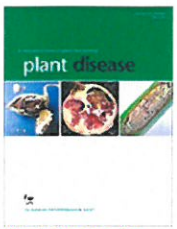

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DISEASE NOTES

First Report of *Phytophthora uniformis* and *P. plurivora* Causing Stem Cankers on *Alnus glutinosa* in Denmark

M. Á. Redondo, Swedish University of Agricultural Sciences, Almas Allé 8, 75007 Uppsala, Sweden; **I. M. Thomsen**, University of Copenhagen, Rolighedsvej 23, 1958, Copenhagen, Denmark; and **J. Oliva**, Swedish University of Agricultural Sciences, Almas Allé 8, 75007 Uppsala, Sweden.

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Phytophthora uniformis (Brasier & S.A. Kirk) Husson, Ioos & Aguayo, comb. nov. is an aggressive pathogen associated with root and collar rot of alder trees (*Alnus* spp.) in Europe (Husson et al. 2015). In summer 2016, symptomatic trees were observed in the banks of a backwater lake area of the river Gurre Å (56°1′46.24″N, 12°27′15.30″E, 29 m above sea level). Several trees displayed chlorosis, reduced leaf size, defoliation, and bleeding cankers on the base of the stem. Inspections upstream near Gurre forest failed in finding other trees with similar crown symptoms, whereas a tree with a bleeding canker was observed downstream (56°3′10.97″N, 12°26′4.40″E, 23 m above sea level). Bark from six trees in the first site and from the tree in the second site was removed from the canker area. As described in Redondo et al. (2015), pieces of 1 cm × 0.5 cm × 1 mm of necrotic cambial tissue from the infection front were plated directly into CMA-PARPBH selective medium. Growing hyphae were transferred onto V8 juice agar medium and incubated at 20°C. Isolates from the first site grew 5 mm/day and formed cottony uniform colonies. After 1 week, spherical smooth-walled oogonia often showing two-celled amphigynous antheridia developed profusely (Ø 39.6 µm; SE = 0.84). Sporangia were ellipsoid and nonpapillate, often with nested proliferation. Colonies from the second site grew 5.5 mm/day, and formed a stellate colony pattern with limited aerial mycelium. Abundant spherical (Ø 29.8 µm; SE = 0.53) oogonia with paragynous antheridia formed after 1 week. Sporangia were commonly ovoid and semipapillate. DNA was extracted from all isolates and the ITS region was amplified using the ITS4 and ITS6f primers. BLAST searches showed 99% similarity with *P. uniformis* sequence no. AF139367.1 for the six isolates from the first site (GenBank accession nos. KX822022 to KX822027), and 99% similarity with *P. plurivora* sequence no. FJ665227.1 for the isolate from the second site (KX822028). As morphological characteristics of the first six isolates corresponded to those of the *P. alni* species complex, primers TRP-PAU-F/-R and RAS-PAM1-F/-R (Ioos et al. 2006) were used to classify them as *P. uniformis*. ITS patterns from Jung and Burgess (2009) were used to classify the *P. plurivora*-like isolate as *P. plurivora*. Pathogenicity of two *P. uniformis* isolates and the *P. plurivora* isolate was confirmed by immersing 30 alder

seedlings, grown in vermiculite for 3 weeks, in a zoospore suspension of each isolate adjusted to 3×10^4 zoospores ml^{-1} for 3 h. Seedlings were later kept in dark Eppendorf tubes in sterile water, and rewatered daily. After 10 days, mortality was 76.6 and 86.6% among those inoculated with *P. uniformis*, and 60% for those inoculated with *P. plurivora*. Surviving seedlings after 10 days displayed wilting symptoms, while seedlings immersed in water were symptomless. The roots of the dead seedlings were plated onto CMA-PARPBH selective medium. Growing hyphae were observed in all plated roots, and were transferred to V8 juice agar medium. The reisolated *Phytophthora* colonies were morphologically similar to *P. uniformis* and *P. plurivora*, fulfilling Koch's postulates. Our finding raises concern about the future health of alder trees in Denmark, a country from which *P. alni* has not yet been reported. However, symptoms of *Phytophthora* disease have been observed on alder since 1995 (Thinggaard 1996). Further monitoring should investigate the extent of the outbreak.

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